Free Radical Biology & Medicine, Vol. 7, pp. 177–178, 1989

0891-5849/89 $3.00 + .00

Forum

ON THE DETECTION OF LIPID HYDROPEROXIDES IN BIOLOGICAL SAMPLES

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In 1987 this Journal published a Forum on the detection of lipid hydroperoxides in biological samples (*FRBM* Vol. 3, No. 5, 1987), which included contributions from seven research groups describing their methods of choice. Since that time we have received several more articles on the same topic, which we present here.

It may prove to be unusual to present two Forums on the same subject, but the detection of the oxidation products of lipids is an extremely important topic. Because radicals generally are very reactive and consequently short-lived, their direct detection is almost always impossible. Thus, indirect methods for detecting radical reactions are required. Even though it is true that electron spin resonance (ESR) in principle can detect radicals directly, the radicals dealt with in biological samples almost always are so reactive and short-lived that the indirect spin trap method must be used. Since the animal being hunted is so fleeting, one must look for its "footprints."

The "footprints" that free radical reactions leave in biological tissue are of a variety of types,[1] but it is remarkable how many involve searching for products of the oxidation of polyunsaturated fatty acids (PUFA). This is partly because of a historical bias, since PUFA oxidation was one of the first consequences of free radical reactions observed in biology, dating to the first observations of the rancidity of milk products and the thiobarbituric acid (TBA) test. Thus, the methods useful for detecting radical "footprints" include:

- ESR (directly, often in frozen samples)
- ESR spin trapping[2–4]
- TBA reactive materials (note these can form from PUFA and also from nucleic acids)[5–9]
- detection of malonaldehyde (MDA) by direct methods (such as HPLC of MDA itself or as its dinitrophenylhydrazone)[10,11]
- detection of other oxidation products from PUFA (such as 4-hydroxynonenal)[12]
- measurement of lipid hydroperoxides[13–19]
- detection of volatile hydrocarbons (ethane, pentane, ethylene)[20–22]
- detection of oxidation products from lipids other than PUFA (e.g., cholesterol)[23,24]
- oxidation of methional, methionine, or 2-keto-4-thiomethylbutanoic acid to ethylene[25,26]
- oxidation of benzoic acid to carbon dioxide (often with radiolabelled carbon dioxide)[27]
- oxidation of phenol, benzoic acid, or aspirin to hydroxylated products[28]
- determination of decreases in antioxidant levels (e.g., decreased GSH, tocopherol, or ascorbate) or of increases in the oxidized products from antioxidants (e.g., tocopherol quinone or the ascorbyl radical)[29]
- detection of oxidized DNA bases (e.g., thymine glycol, 8-hydroxydeoxyguanosine)[30]
- detection of oxidized products from proteins (e.g., methionine sulfoxide from methionine) or of proteins oxidized to carbonyl-containing products that then react with hydride-reducing agents[31,32]
- detection of adducts of DNA bases (e.g., by enzymatic hydrolysis post-labeling using ^{32}P)[33,34]
- chemiluminescence methods[35,36]

This is an impressive list indeed, but somehow one is left with a feeling that the "perfect, universal" method is not yet in hand. Maybe there is no perfect method, applicable for all situations. For example, smokers do not show elevated levels of TBA reactive substances (TBARS) in their plasma[1,29] but do show elevated ethane/pentane, suggesting that one must search for oxidative changes in the organ at risk.[22] Similarly, smokers have normal plasma vitamin E, but decreased levels in lung lavage.[29]

It also is unfortunate that a single group does not more often publish the results of several of these tests, since, as indicated above, even a lack of agreement can be revealing. And agreement using different tests can serve to establish the validity and reliability of different indexes of oxidative stress.

Perhaps one of the greatest needs in the field now is the availability of a noninvasive test to probe the oxidative stress status (OSS) of humans. If we could measure OSS, we could ask the question: In what dis-

eases is OSS elevated? And then we could ask the $64 questions: Do antioxidants prevent this elevation. And, if so, does this result in some protection against pathologies or diseases?

It is our hope that this Forum will focus attention of our readers on these and similar questions, and that you will find these articles useful and challenging. As always, your comments and suggestions are most welcome.

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